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ORIGINAL ARTICLE

Plasma homocysteine level is unrelated to long-term cardiovascular events in patients with previous percutaneous coronary intervention

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Summary
Objectives: It is unclear whether plasma homocysteine (Hcy) level affects long-term outcomes in patients with previous percutaneous coronary intervention (PCI). Accordingly, we investigated the association of plasma Hcy level with long-term major adverse cardiovascular events (MACEs), especially with recurrence of angina pectoris (AP) or new myocardial infarction (MI) in patients with previous PCI.
Methods: A total of 231 patients with previous (>12 months) PCI were followed up for a median period of 49 months. The primary end point was recurrence of AP or new MI. The secondary end points were MACEs (cardiovascular death, recurrence of AP, new MI, revascularization therapy, hospitalization for heart failure, or stroke).
Results: During the follow-up period, 35 patients (15.2%) had a primary end point, and 58 (25.1%) had a secondary end point. A univariate analysis by a Cox proportional hazards regression model showed that plasma Hcy level was not associated with the primary (hazard ratio [HR] 1.13, 95% confidence interval [CI] 0.41—3.08, $p = 0.82$) and secondary (HR 1.60, 95% CI 0.75—3.42, $p = 0.23$) end points. The adjustment for other clinical variables did not alter the results.
Conclusions: Plasma Hcy level appears to be unrelated to recurrent AP, new MI, and long-term MACE within coronary artery disease patients with previous PCI.

Introduction

Homocysteine (Hcy) is a sulfhydryl-containing amino acid derived from the demethylation of methionine, one of the essential amino acids. MuCully firstly proposed a link between hyperhomocysteinemia and atherosclerotic diseases, based on the findings of autopsies of children with homocystinuria, an inborn error in methionine metabolism [1]. Since a report [2] describing a relationship between hyperhomocysteinemia and coronary artery disease (CAD), the relationship

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has been paid much attention [3]. Experimental studies have suggested that Hcy may promote atherosclerosis through several mechanisms including endothelial dysfunction [4], oxidation of low-density lipoprotein [5,6], the activation of inflammatory pathways [7], smooth muscle cell proliferation [4,7], and the activation of coagulation factors and platelets [4,7].

Previous studies have reported that the elevated level of plasma Hcy is associated with a higher incidence of restenosis after percutaneous coronary intervention (PCI), although conflicting results exist [8,9], and that patients with an elevated level of plasma Hcy have an adverse short-term outcome after PCI [10,11]. However, it is unclear whether the plasma Hcy level affects long-term outcomes in patients with previous PCI. Accordingly, we sought to clarify the association of plasma Hcy level with long-term major adverse cardiovascular events (MACEs), especially with recurrence of angina pectoris (AP) or new myocardial infarction (MI) in patients with previous PCI.

Methods

Study population and data collection

Between August 1998 and March 2000, 231 patients (184 men and 47 women, aged 66 ± 8 years) who met the following criteria were enrolled in this study: (1) patients with previous (>12 months) PCI for at least one stenotic lesion in the native coronary arteries, and (2) no restenosis of the target lesions after PCI, which was confirmed by follow-up coronary angiography. Exclusion criteria included age >80 years, acute coronary syndrome within the preceding 12 months, severe symptoms of congestive heart failure (New York Heart Association functional class IV), concomitant idiopathic myocardial diseases and vulvular heart diseases requiring a cardiac operation, life-threatening arrhythmias, hemodialysis or peritoneal dialysis, malignancy, or severe chronic diseases. The study protocol was approved by the ethics committee at our institution, and informed consent was obtained from each patient before the study.

Venous blood samples were drawn after an overnight fast, and serum levels of creatinine, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglycerides were measured. Serum creatinine levels were measured by an enzyme method. Plasma total Hcy levels were determined using a high-performance liquid chromatography assay. Hyperhomocysteinemia was defined as a plasma Hcy level of >15 μmol/L and was classified into the following 3 groups: mild ($15 < Hcy \leq 30$ μmol/L); moderate ($30 < Hcy \leq 100$ μmol/L); and severe (Hcy > 100 μmol/L) [12]. The estimated glomerular filtration rate (eGFR) was calculated using the modification of diet in renal disease formula modified by the Japanese Society of Nephrology [13] and was expressed as per 1.73 m^2 of the body surface area. Chronic kidney disease (CKD) was defined as an eGFR < 60 mL/min/1.73 m^2 [14]. A 75-g oral glucose tolerance test was performed if patients were not treated with oral hypoglycemic drugs and/or insulin. The patients whose fasting plasma glucose level ≥126 mg/dL and/or plasma glucose level ≥200 mg/dL at 2 h after the glucose load were diagnosed as having diabetes mellitus (DM).

Table 1 Baseline characteristics.

Number of patients	231
Age (years)	66 ± 8
Male	184 (79.7)
BMI (kg/m^2)	24 ± 3
Current smoker	45 (19.5)
Family history of CAD	71 (30.7)
Previous MI	126 (54.5)
Multivessel disease	54 (23.4)
DM	90 (39.0)
Systolic blood pressure (mmHg)	118 ± 14
Diastolic blood pressure (mmHg)	68 ± 10
LDL cholesterol (mg/dL)	115 ± 28
HDL cholesterol (mg/dL)	45 ± 12
Triglycerides (mg/dL)	121 ± 58
Hcy (μmol/L)	9.0 (7.5—11.3)
Estimated GFR (mL/min/1.73 m^2)	63 ± 17
CKD	101 (43.7)
LVEF (%)	60 ± 14
Methods of PCI	
Balloon angioplasty	137 (59.3)
Bare-metal stent	82 (35.5)
Drug-eluting stent	0 (0)
Rotablation	12 (5.2)
Medications	
Nitrates	177 (76.6)
Diuretics	50 (21.6)
Calcium antagonists	178 (77.1)
ACE inhibitors	65 (28.1)
Angiotensin II receptor blockers	38 (16.5)
Beta blockers	52 (22.5)
Statins	164 (71.0)

Values are expressed as mean ± S.D., median (first—third quartiles), or number (%). BMI, body mass index; CAD, coronary artery disease; MI, myocardial infarction; DM, diabetes mellitus; LDL, low-density lipoprotein; HDL, high-density lipoprotein; Hcy, homocysteine; GFR, glomerular filtration rate; CKD, chronic kidney disease; LVEF, left ventricular ejection fraction; PCI, percutaneous coronary intervention; ACE, angiotensin-converting enzyme.

Left ventricular ejection fraction was determined by echocardiography and was calculated using a modification of Simpson's rule [15].

The primary and secondary end points

The primary end point was recurrence of AP or new MI. The secondary end points were MACEs, including cardiovascular death, recurrence of AP or new MI, revascularization therapy, hospitalization for heart failure, and stroke. Cardiovascular deaths included any death for which there was no clearly documented non-cardiovascular cause.

Statistical analysis

Continuous variables are presented as mean ± S.D. or median (first—third quartiles). Categorical data were analyzed by the chi-square test. Comparisons of continuous data were performed using the unpaired *t*-test. The event-free rates for the primary and secondary end points between patients with a plasma Hcy level ≥ the median value and those with a plasma Hcy level < the median value were estimated using the Kaplan—Meier method, and the event-free curves were then compared using the log-rank test. Univariate and multivariate analyses using a Cox proportional hazards regression model were performed to determine the predictors of the primary and secondary end points. A multivariate analysis was performed using variables that showed $p \leq 0.2$ in the univariate analyses. Logarithmically transformed Hcy values were used in these analyses in order to ensure a normal distribution. A p-value < 0.05 was considered to be statistically significant. Data analysis was carried out using SPSS 12.0J for Windows (SPSS Inc., Tokyo, Japan).

Results

The median follow-up period was 49 months. Baseline characteristics of the study population are shown in Table 1. The prevalence of DM, previous MI, multivessel disease, and CKD was 39.0%, 54.5%, 23.4%, and 43.7%, respectively. The low-density lipoprotein cholesterol level was 115 ± 28 mg/dL. Nitrates, diuretics, calcium antagonists, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, beta blockers, and statins were administered to 76.6%, 21.6%, 77.1%, 28.1%, 16.5%, 22.5%, and 71.0% of the study population, respectively. Balloon angioplasty, bare-metal

Table 2 Baseline characteristics of patients with a plasma Hcy level ≥9.0 μmol/L (the median value) and those with a plasma Hcy level <9.0 μmol/L.

Variables	Hcy < 9.0 μmol/L (n = 115)	Hcy ≥ 9.0 μmol/L (n = 116)	p-Value
Age (years)	66 ± 7	67 ± 8	0.83
Male	85 (73.9)	99 (85.3)	0.03
BMI (kg/m^2)	25 ± 3	24 ± 3	0.07
Current smoker	23 (20.0)	22 (19.0)	0.84
Family history of CAD	40 (34.8)	31 (26.7)	0.18
Previous MI	51 (44.3)	75 (64.7)	<0.01
Multivessel disease	25 (21.7)	29 (25.0)	0.55
DM	48 (41.7)	42 (36.2)	0.38
Systolic blood pressure (mmHg)	118 ± 13	118 ± 15	0.79
Diastolic blood pressure (mmHg)	69 ± 9	68 ± 10	0.64
LDL cholesterol (mg/dL)	117 ± 27	114 ± 29	0.37
HDL cholesterol (mg/dL)	46 ± 11	44 ± 12	0.22
Triglycerides (mg/dL)	118 ± 49	124 ± 66	0.45
Estimated GFR (mL/min/1.73 m^2)	69 ± 16	58 ± 16	<0.01
CKD	36 (31.3)	65 (56.0)	<0.01
LVEF (%)	63 ± 13	57 ± 15	0.03
Medications			
Nitrates	88 (76.5)	89 (76.7)	0.97
Diuretics	19 (16.5)	31 (26.7)	0.07
Calcium antagonists	90 (78.3)	88 (75.9)	0.66
ACE inhibitors	28 (24.3)	37 (31.9)	0.20
Angiotensin II receptor blockers	18 (15.7)	20 (17.2)	0.74
Beta blockers	25 (21.7)	27 (23.3)	0.78
Statins	84 (73.0)	80 (69.0)	0.49

Data are presented as mean ± S.D. or number (%). For abbreviations, see Table 1.

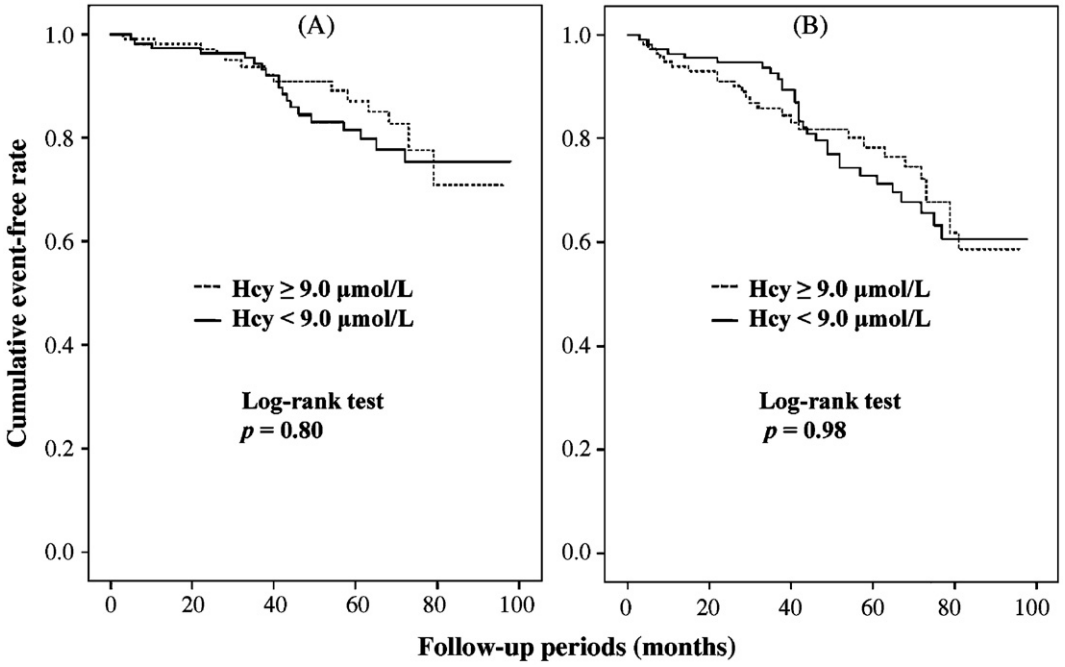

Figure 1 Kaplan—Meier curves of cumulative event-free rates for the primary (A) and secondary (B) end points between patients with a plasma homocysteine (Hcy) level of ≥9.0 μmol/L and those with a plasma Hcy level of <9.0 μmol/L.

stenting, and rotablation were undergone in 59.2%, 35.5%, and 5.2% of the study population, respectively. No patients underwent drug-eluting stenting.

The median Hcy level was 9.0 μmol/L, and 210 patients (90.9%) had a normal plasma Hcy level. Mild and moderate hyperhomocysteinemia were observed in 20 (8.7%) and 1 (0.4%) of the patients studied, respectively. None of the patients had severe hyperhomocysteinemia. Table 2 shows baseline characteristics of patients with a plasma Hcy level ≥9.0 μmol/L and those with a plasma Hcy level <9.0 μmol/L. Patients with a plasma Hcy level ≥9.0 μmol/L had higher frequencies of male gender, previous MI, and CKD than those with a plasma Hcy level <9.0 μmol/L. The former had a lower eGFR and LVEF than the latter.

Table 3 shows the incidence of the primary and secondary end points during the follow-up period. Thirty-five patients (15.2%) and 58 patients (25.1%) had primary and secondary end points, respectively. Of the latter patients, 5 (2.2%) died of cardiovascular causes, 9 (3.9%) were hospitalized for heart failure, 38 (16.5%) underwent revascularization therapy, and 5 (2.2%) had a stroke. The Kaplan—Meier curves showed no significant differences in the event-free rates of primary and secondary end points between patients with a plasma Hcy level of ≥9.0 μmol/L and those with a plasma Hcy level of <9.0 μmol/L (Fig. 1).

Table 4 shows the results of univariate and multivariate analyses by a Cox proportional hazards model performed to determine the predictors of the primary and secondary end points. On univariate analyses, log Hcy was not associated with the primary (hazard ratio [HR] 1.13, 95% confidence interval [CI] 0.41—3.08, $p = 0.82$) and secondary (HR 1.60, 95% CI 0.75—3.42, $p = 0.23$) end points. The adjustment for other clinical variables shown in Table 4 did not alter the results (primary end point: HR 1.15, 95% CI 0.34—3.90, $p = 0.82$; secondary end point: HR 1.35, 95% CI 0.54—3.36, $p = 0.52$). On univariate analyses, DM was significantly associated with the primary and secondary end points, and CKD was significantly

Table 3 Incidence of the primary and secondary end points.

Primary end point	35 (15.2)
Recurrence of AP or new MI	35 (15.2)
Secondary end point	58 (25.1)
Recurrence of AP or new MI	35 (15.2)
Cardiovascular death	5 (2.2)
Hospitalization for heart failure	9 (3.9)
Revascularization therapy	38 (16.5)
Stroke	5 (2.2)

Data are presented as number (%). AP, angina. For another abbreviation, see Table 1.

Table 4 Univariate and multivariate analyses using a Cox proportional hazards model in order to determine the predictors of the primary and secondary end points.

Variables	Primary end point				Secondary end point			
	Univariate		Multivariate		Univariate		Multivariate	
	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
Age (years)	1.01 (0.97—1.06)	0.61			1.02 (0.99—1.06)	0.22		
Male	1.17 (0.49—2.82)	0.73			1.05 (0.54—2.02)	0.89		
BMI (kg/m^2)	1.08 (0.98—1.19)	0.14	1.06 (0.96—1.18)	0.24	1.04 (0.96—1.13)	0.35		
Current smoker	1.21 (0.64—1.21)	0.22			1.07 (0.57—2.03)	0.83		
Family history of CAD	0.96 (0.46—2.00)	0.91			0.83 (0.46—1.49)	0.52		
Previous MI	0.99 (0.51—1.93)	0.98			0.92 (0.55—1.55)	0.77		
Multivessel disease	1.38 (0.66—2.88)	0.39			1.21 (0.67—2.17)	0.53		
DM	2.28 (1.17—4.45)	0.02	2.03 (1.02—4.03)	0.04	2.19 (1.30—3.69)	<0.01	2.12 (1.23—3.65)	<0.01
Systolic blood pressure (mmHg)	0.99 (0.97—1.02)	0.62			1.01 (0.97—1.03)	0.37		
Diastolic blood pressure (mmHg)	0.99 (0.96—1.03)	0.65			1.00 (0.97—1.03)	0.83		
LDL cholesterol (mg/dL)	1.00 (0.99—1.02)	0.50			1.01 (0.99—1.02)	0.29		
HDL cholesterol (mg/dL)	1.00 (0.97—1.03)	0.80			0.99 (0.97—1.01)	0.28		
Triglycerides (mg/dL)	1.00 (0.99—1.01)	0.14	1.00 (0.99—1.01)	0.48	1.00 (0.99—1.01)	0.29		
Log Hcy (μmol/L)	1.13 (0.41—3.08)	0.82			1.60 (0.75—3.42)	0.23		
CKD	1.18 (0.60—2.30)	0.64			1.79 (1.07—3.01)	0.03	1.77 (1.04—3.01)	0.04
LVEF (%)	1.00 (0.98—1.03)	0.76			0.99 (0.98—1.01)	0.41		
Nitrates	0.93 (0.43—1.98)	0.84			1.38 (0.72—2.67)	0.33		
Diuretics	0.89 (0.39—2.03)	0.78			1.24 (0.69—2.22)	0.48		
Calcium antagonists	1.40 (0.48—4.01)	0.53			1.18 (0.55—2.51)	0.67		
ACE inhibitors	1.14 (0.56—2.33)	0.72			1.52 (0.89—2.58)	0.12	1.17 (0.67—2.04)	0.59
Angiotensin II receptor blockers	0.99 (0.23—4.32)	0.99			1.35 (0.52—3.51)	0.54		
Beta blockers	1.65 (0.77—3.54)	0.20	1.59 (0.73—3.47)	0.24	1.48 (0.81—2.72)	0.20	1.30 (0.70—2.39)	0.40
Statins	1.19 (0.56—2.53)	0.66			1.41 (0.76—2.61)	0.28		

HR, hazard ratio; CI, confidence interval. For other abbreviations, see Table 1

associated with the secondary end point. On a multivariate analysis, DM was an independent predictor of the primary and secondary end points (HR 2.03, 95% CI 1.02—4.03, $p = 0.04$, and HR 2.12, 95% CI 1.23—3.65, $p < 0.01$, respectively), and CKD was an independent predictor of the secondary end point (HR 1.77, 95% CI 1.04—3.01, $p = 0.03$).

Discussion

The major findings of the present study are as follows: in patients with previous PCI, (1) the plasma Hcy level was not associated with recurrence of AP or new MI and long-term MACEs; (2) DM was an independent predictor of recurrence of AP or new MI and long-term MACEs; and (3) CKD was an independent predictor of long-term MACEs.

Plasma Hcy levels and the long-term outcome in patients with previous PCI

Previous studies have reported that patients with an elevated level of plasma Hcy have an adverse short-term outcome after PCI [10,11]. However, it has not been clarified whether plasma Hcy level affects long-term outcomes in patients with previous PCI. The present study found no associations of plasma Hcy levels with long-term adverse outcomes in patients with previous PCI.

Early cross-sectional and retrospective studies have indicated a positive association between plasma Hcy levels and CAD [3]. However, recent prospective studies have indicated that plasma Hcy levels have only a weak association with CAD [16,17]. A recent meta-analysis [18] showed that the odds ratios for CAD in a 5-μmol/L increment of plasma Hcy level were 1.06 (95% CI 0.99—1.13) in cohort studies, 1.23 (95% CI 1.07—1.41) in nested case-control studies, and 1.70 (95% CI 1.50—1.93) in case-control studies. More recently, the prospective epidemiological study of myocardial infarction study showed no association between plasma Hcy levels and future coronary events including nonfatal MI, death from CAD, or angina pectoris in 10,593 men without CAD [19]. Furthermore, prospective large clinical trials could not find that Hcy-lowering therapy with folic acid, vitamin B_{12}, and vitamin B_6 reduces the incidence of future cardiovascular events in patients with acute MI [20] and stable CAD [21]. Thus, the status of plasma Hcy level as a risk factor for CAD has not yet been established [22].

Renal function is well known to affect plasma Hcy levels [23]. Muntner et al. [24] reported that the frequencies of hyperhomocysteinemia were 32.9% of persons with a GFR of 15—59 mL/min/1.73 m^2 and 6.1% of persons with a GFR of 60—89 mL/min/1.73 m^2. In the present study, patients with a plasma Hcy level ≥9.0 μmol/L had a lower eGFR than those with a plasma Hcy level <9.0 μmol/L. Therefore, renal function should be taken into consideration when investigating the relationship between plasma Hcy levels and cardiovascular events. Menon et al. [25] reported that the plasma Hcy level was not associated with all-causes or cardiovascular deaths after the adjustment for GFR in 804 patients with CKD. It is well known that CKD is an important risk factor for future cardiovascular events [26]. The present study also demonstrated that CKD was an independent predictor of long-term MACEs in patients with previous PCI. The association between plasma Hcy levels and future cardiovascular events having been previously reported may have indirectly represented an association between renal dysfunction and future cardiovascular events. Further studies are needed to clarify whether hyperhomocysteinemia is indeed associated with adverse cardiovascular outcomes independent of renal function.

DM and the long-term outcome in patients with previous PCI

Previous studies have demonstrated that patients with DM have a 2—3-fold risk of future cardiovascular events than non-diabetics [27,28] and that diabetic patients have adverse short- and long-term outcomes after PCI [29—31]. The present study also showed that DM is an independent predictor of long-term adverse outcomes in CAD patients with previous PCI. Several mechanisms for an association between DM and the adverse outcomes are considered. Coronary atherosclerotic disease is more advanced in diabetic patients than non-diabetic patients [31—33], and diabetic patients with CAD often have multiple vulnerable plaques [34] that would be associated with future acute coronary syndrome. Pathophysiologically, in diabetic patients, the metabolic abnormalities including hyperglycemia, increased free fatty acids, and insulin resistance provoke molecular mechanisms that alter the function and structure of blood vessels [35], leading to the progression of atherosclerosis and subsequently increasing risk of adverse cardiovascular events.

Strengths of the present study

The strengths of the present study are as follows. First, the presence or absence of stenotic lesions

in the coronary arteries was evaluated by coronary angiography in all patients. Second, the present study included only stable CAD patients with previous PCI who did not developed restenosis of the target lesion after PCI, which was confirmed by follow-up coronary angiography. Third, the target lesion for recurrent AP or new MI was confirmed by coronary angiography in all patients except those with sudden death.

Limitations of the present study

The present study has certain limitations. First, the sample size of the present study was relatively small. Second, in the present study, the prevalence of hyperhomocysteinemia was only 9.1%, and 95% of patients with hyperhomocysteinemia had mild hyperhomocysteinemia. This might be partly because patients with a serum creatinine level >2.5 mg/dL were not included in the present study: we do not generally perform coronary angiography or PCI for those patients who do not undergo chronic dialysis. As renal insufficiency is an important risk factor for the elevated level of plasma Hcy [24], a lack of patients with a serum creatinine level >2.5 mg/dL may have been associated with a low prevalence of hyperhomocysteinemia in the present study. Finally, although it was recently reported that the plasma free Hcy level but not the plasma total Hcy level is an independent risk factor for recurrent cardiovascular events in patients hospitalized for acute coronary syndrome [36], we did not measure the plasma free Hcy level.

Conclusions

The plasma Hcy level appears to be unrelated to recurrent AP, new MI, and long-term MACEs in CAD patients with previous PCI.

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